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LA-UR -89-850

Received by OSTI

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LA-UR--89-850

DE89 009393

TITLE OPTICAL TRAPPING, CELL MANIPULATION AND ROBOTICS

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SUBMITTED TO Proceedings of the SPIE Symposium on Medical Applications of Lasers and Optics

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To appear in Proceedings of the SPIE Symposium on the Medical Applications of Lasers and Optics, Los Angeles, 1989.

Optical Trapping, Cell Manipulation, and Robotics

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ABSTRACT

A new type of analytical and preparative cytometric instrument was developed. The instrument combines image analysis and machine vision with single cell and chromosome manipulation by means of optical trapping. A proof-of-principle instrument, OCAM, has the ability to locate and analyze biological particles inside an enclosed manipulation chamber, as well as the ability to move and position particles according to preprogrammed protocols. Preliminary results and potential biological applications of such a microrobot are discussed.

1. INTRODUCTION

The last twenty years have seen the development of two major classes of high-performance analytical instruments. These are: (i) video microscope-based image processing workstations; and (ii) flow cytometers. Traditionally, the two classes have been characterized by widely differing capabilities and, therefore, applications. Thus, image processing microscopes have been capable of analyzing biological particles at low speed but with high resolution, whereas flow cytometers have been capable of zero-resolution imaging at very high speeds. Moreover, flow cytometers have naturally lent themselves to preparative applications (flow sorting), while imaging instruments, fitted with mechanical micromanipulators, have seen only limited preparative use.

Current developments in the field of cytometric instrumentation suggest that the distinction between imaging and flow instruments is beginning to disappear. Indeed, one-dimensional imaging (scanning) flow cytometers are already in use, and several imaging flow cytometers are being developed which will provide images of moderate but useful resolution. The discovery of optical trapping by Ashkin [1] in the late 60s, and especially the recent technical developments in optical trapping as applied to biological problems [2,3,4], have opened up a completely new range of applications for imaging instruments as preparative and manipulation devices. The robotic optical trapping manipulator described in this article is a good example of the spectrum of capabilities inherent in instrumentation based on optical trapping.

For many reasons, optical trapping as a means for manipulating microscopic biological particles, is far superior to mechanical micromanipulators. Indeed, optical trapping can easily be achieved in a *completely enclosed chamber*, thus preventing crosscontamination between the sample and the laboratory environment and allowing one to control very accurately and over extended periods of time the physical and chemical parameters of the biological sample. Furthermore, the absence of mechanical devices makes possible the use of manipulation chambers with *very fine structural details*, such as microscopic compartments and interconnecting channels. The presence of such chamber structures opens up new possibilities for the separation and processing of individual biological particles.

The potentially complex structure of the manipulation chamber, together with the ability of the instrument to image, analyze, separate, and position biological particles inside the chamber, can only be used efficiently if appropriate analysis and control software is available to replace a human operator. When endowed with such software, an optical manipulator becomes a true microscopic robot, in the sense that it processes visual (video) information to determine the detailed way in which to execute some predetermined task. It is particularly this combination of imaging, optical trapping, machine vision, and robotics that makes our instrument the first of what we see as a new class of analytical and preparative cytometric instrumentation.

This article begins with a brief discussion of the physics and history of optical trapping. This is followed by a discussion of our current instrument, OCAM, and of its user interface and control software. We present preliminary results demonstrating the ability of the instrument to manipulate live cells and chromosomes, following which we discuss current and future applications of a robotic optical trapping cell manipulator in biology and biotechnology.

2. OPTICAL TRAPPING: HISTORY AND PHYSICS IN BRIEF

Light pressure was first mentioned by the 17th century German astronomer Johannes Kepler, who suggested that it caused comets' tails to point away from the Sun. Although the theory of the pressure exerted by light on spherical particles was published by Debye [5] around the turn of the century, it was not until the late 60s that the pressure exert-

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ed by light on small particles became a phenomenon with practical applications. Indeed, Arthur Ashkin and collaborators at Bell Laboratories discovered that a finely collimated laser beam could not only propel, but also *trap* small particles [1]. It was precisely the intrinsic stability of the optical trap, which thus did not require external stabilizing mechanisms, that made optical trapping practical.

For almost twenty years, optical trapping was only used in a few, nonbiological applications. These included the levitation of small particles in light scattering studies [6], and, more importantly, the deflection of neutral atom beams[7] and the trapping of atoms for Doppler shift-free spectroscopic measurements [8]. It was again Ashkin who, in 1987, reported the trapping of live bacteria and of viruses [2]. This paper can be regarded as the starting point for the biological applications of optical trapping, as it stimulated interest in, and activity towards the development of both instrumentation and biological applications. Ashkin also reported that yeast cells divide inside the optical trap, and that organelles could be manipulated inside certain protozoa [3].

We demonstrated the first automated cell sorter based exclusively on optical trapping [4,9]. This instrument employed weakly focused laser beams to propel single particles through an analysis region, where certain optical properties of the cells were measured. The instrument subsequently made a sorting decision, following which it controlled the intensity of a deflection beam in order to extract from the propulsion beam and separate selected particles. The second-generation instrument we have developed [10,11] is the first microrobot based on optical trapping, which is capable of analyzing, separating, and further processing selected biological particles.

The optical trapping of particles larger than the trapping wavelength can be described to a good approximation in purely geometric optics terms [5]. Following Ashkin [11], one considers a transparent, spherical particle with an internal refractive index higher than that of the surrounding medium and a ray incident on the particle (Fig. 1). If one considers photons travelling "along" the refracted ray, then, at the point of refraction, the momentum of a photon changes from the initial momentum, $\mathbf{p_0}$, to the momentum corresponding to the new direction of the motion of the photon, $\mathbf{p_1}$. The change in the momentum of one scattered photon is represented in the drawing by $\Delta\mathbf{p}$, and, because of the conservation of momentum, the particle receives a momentum equal to $-\Delta\mathbf{p}$ for each scattered photon. By muluplying the momentum transferred to the particle by one photon by the number of photons scattered per unit time, one obtains the net force acting on the particle at the point where the ray is refracted. This force obviously has the same orientation as the transferred momentum, $-\Delta\mathbf{p}$.

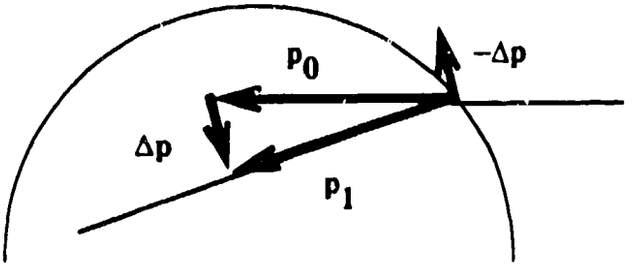

Figure 1. Refraction of a light ray by a spherical surface, and changes in momenta.

Figure 2 shows two rays that are symmetrical relative to the center of the particle, as well as the forces at the points where the two rays are refracted. The force $\mathbf{F_a}$ produced by the refraction of ray a is smaller than the force $\mathbf{F_b}$ produced by the refraction of ray b, as the latter is closer to the beam axis and therefore the flux of photons along it is higher. One can see from the diagram that the axial components of the two forces point in the same direction (in the direction of propagation of the beam), and, therefore, they both tend to propel the particle along the beam. At the same time, the radial components of the two forces point in opposite directions, with the radial force due to the refraction of ray b dominating. Thus, the net radial

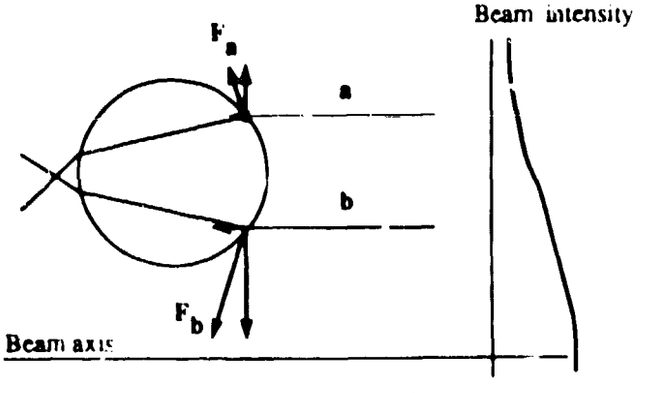

Figure 2. Spherical particle in a Gaussian laser beam

force always points toward the beam axis, which thus appears to *attract* the particle. This is the basis for the radial, two-dimensional, optical trapping effect.

While radial trapping is easy to achieve, axial trapping requires the use of strongly convergent beams, and, therefore, very short focal length focusing lenses. Alternatively, axial trapping can be achieved through the use of two coaxial, counterpropagating laser beams. In the latter configuration, the radial forces due to the two beams add up, whereas the axial forces cancel each other out at a point between the waists of the two beams. Thus, a full three-dimensional (3-D) trap can be obtained with more weakly focused beams. We adopted this method as it allows one to use relatively low magnification lenses with large working distances.

3. OCAM - A ROBOTIC OPTICAL TRAPPING CELL MANIPULATOR

3.1. Hardware

As mentioned in the previous section, the optical manipulator we developed uses two coaxial, counterpropagating beams to produce a 3-D trap. A simplified diagram of the instrument optics is shown in Fig. 3. The beam from the trapping laser is split into two beams of equal intensity, which are then reflected by dichroic mirrors and focused by

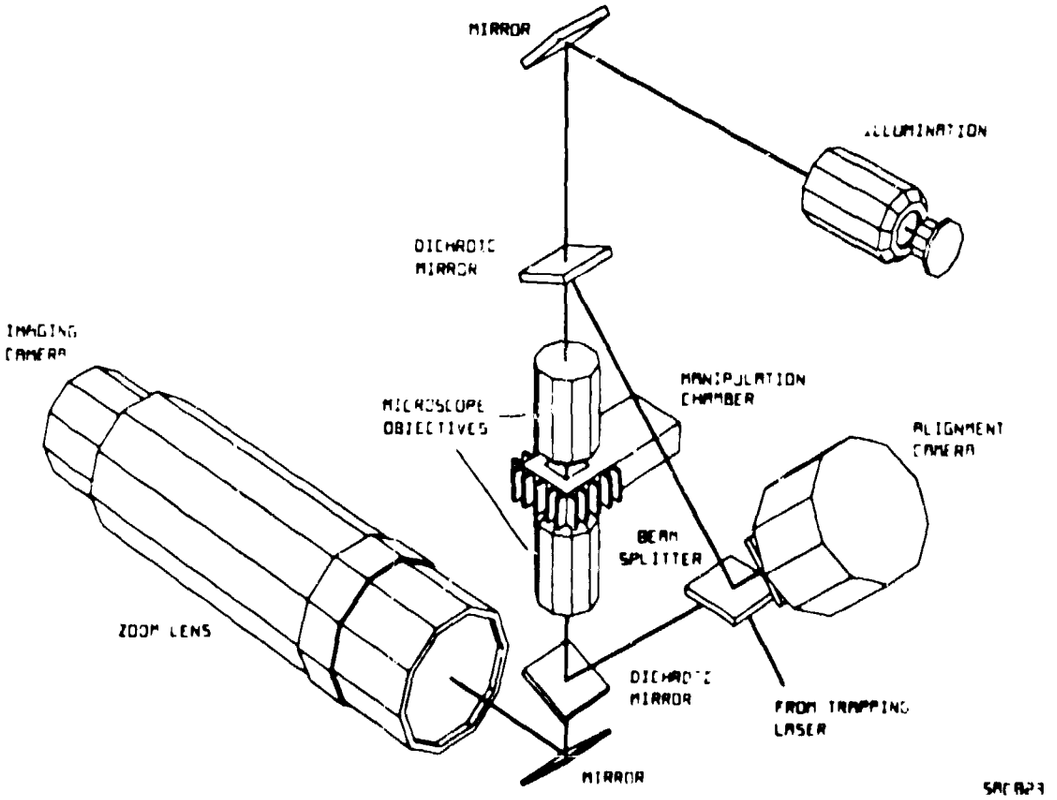

Figure 3. Simplified diagram of the instrument optics.

microscope objectives as coaxial, counterpropagating beams. The emerging beams, which have travelled once around the optical system, are imaged by a dedicated video camera. The video image is used for aligning the optical system and ensuring that the two trapping beams are indeed coaxial. The same image is also used for adjusting the distance between the two beam waists.

The imaging system shares some of the optical elements with the trapping system. Thus, the dichroic mirrors that reflect the trapping beams allow the imaging wavelengths to pass through, whereas the objectives used for focusing the trapping beams are also used for illumination (upper objective) and imaging (lower objective). A commercial zoom lens is used as a video eyepiece and forms an image on the sensor of a video camera. The imaging system is completed by a frame grabber and image processor (Data Translation, Marlboro, MA) that supplies digitized and preprocessed im-

ages to the control computer (VAXstation II GPX, Digital, Maynard, MA).

The instrument has several control elements that bear on the optical trap. These are precision servopositioners that control trapping beam alignment, as well as the position of the trap relative to the manipulation chamber. In our instrument, the trapping beams are stationary, and it is the position of the chamber itself that can be adjusted. This greatly simplifies the optics, and, more importantly, allows the relative position of the trap to be controlled over large distances (typically 10mm range for all three position coordinates). The ability to move the trap over large distances in the plane of the chamber is particularly important when chambers with multiple compartments are used.

The optical manipulation chamber is an essential part of the instrument. Indeed, the characteristics of the chamber determine to a large extent the range of biological applications of the instrument. We have developed a chamber design that consists of a thin central section sandwiched between two windows of good optical quality. The central section contains microscopic compartments and interconnecting channels which are either laser-machined into thin (120 μm) stainless steel shim stock, or photoetched into a photosensitive ceramic. The design of the ceramic center section is shown in Fig. 4a, with an enlarged view of the chamber compartments and interconnecting channels shown in Fig. 4b. One of the windows is a 170μm-thick glass coverslip, while the other window is a 1mm-thick glass slide with holes in which the external ports are mounted. The thin window lies on the imaging side. The thick window also plays a structural role by supporting the external ports and giving rigidity to the chamber. The sandwich design allows one to produce chambers with complex internal structures and with uniform overall shape. This design also maximizes heat transfer between the chamber and its holder, thus allowing accurate temperature control and, if required, fast temperature changes.

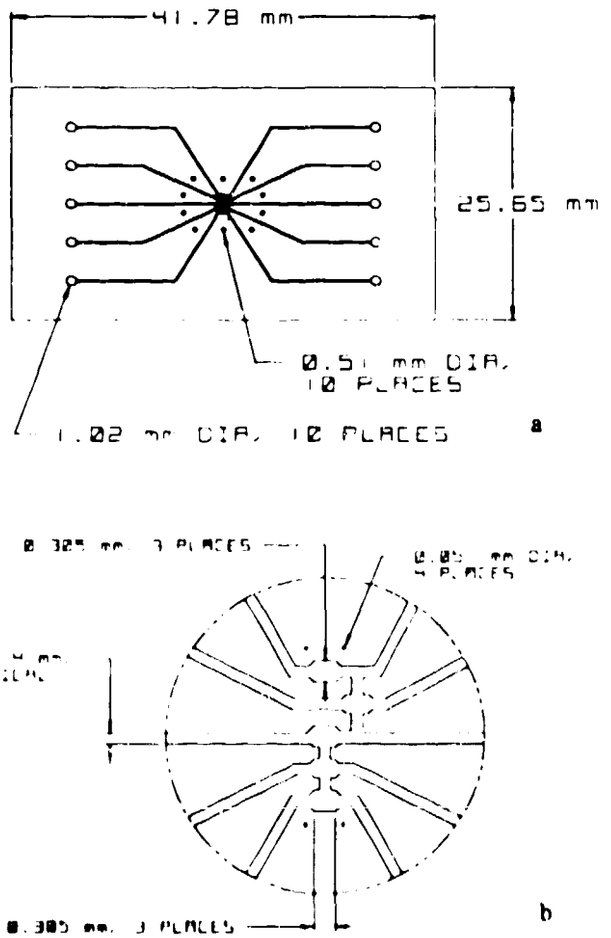

Figure 4. Diagram of the chamber center section (a), and enlarged view of the compartments and interconnecting channels (b)

The multiple compartments in this chamber and the interconnecting channels will allow us to perform complex experiments. The compartments are, in fact, the microscopic equivalent of test tubes between which the instrument can transfer cells and other biological particles. Furthermore, the channels connecting the compartments with the external ports allow the composition of the suspension medium in the compartments to be modified at will. Some of the compartments (the two smaller ones in Fig. 4b) can be filled with air, thus isolating the main compartments of the chamber from each other.

3.2. User Interface

All control elements of the instrument, including fluidics and the imaging system, are driven by the control computer. The operator interacts with the computer through a graphics interface and a mouse. The graphics interface displays a set of virtual instruments with control elements that can be acted upon by the operator through the mouse.

The operator can control trap position in three ways. The first of these employs a virtual 3-D joystick through which the three components of the trap velocity can be controlled. This mode of control is particularly useful when rapidly scanning the sample. A second control mode uses a map of the chamber that is displayed on the computer screen. The positions of the trap and imaged area are displayed on the map, together with the three coordinates of the trap. The operator can use the mouse to draw on the map a target point. Alternatively, a sequence of points defin

ing a complex polygonal path can be drawn on the map. Once the path has been drawn, the instrument moves the trap along the preprogrammed path. This mode is particularly useful when the trap must be moved across a chamber area that is larger than the imaged region. Finally, a binary version of the video image is also displayed on the control screen. After calibrating the video system, the operator can draw a polygonal path over the video image. This mode of operation is important when small adjustments in the position of the trap are required.

4. IMAGE ANALYSIS AND MACHINE VISION

The image analysis problem for the optical manipulator is the same as that for any cytometric image analysis system, namely to identify and separate individual biological particles and to compute various morphometric parameters for each particle. More interesting is the machine vision and motion control problem, which follows form the robotic aspect of the instrument. Indeed, the most important task of the instrument is to transport selected particles reliably between two points inside the chamber. In this context, reliability means (i) avoiding collisions both with the chamber walls and with other particles; (ii) detecting the loss of the trapped particle; and (iii) detecting the fortuitous trapping of unwanted particles. Whereas the latter two points are implemented relatively easily by monitoring the pixels in the trap region, the former is more difficult. The control software on our instrument performs the collision avoidance task in two steps. The first step consists of determining a rough path that does not collide with the chamber walls (Fig.5a). This is done by using a stored map of the chamber compartments and interconnecting channels. In a second step, the information generated by the image analysis software about objects present in the current video frame is used to compute a clear path leading from the current position of the trap in the general direction of the rough path (Fig. 5b). As the trap approaches the edge of the frame, a new frame is analyzed in the same way, and the whole process is repeated until the target point is reached. This procedure results in a sequence of overlapping frames that covers the rough path and that contains the actual, clear, path. Obviously, information about the geometry of the trap is also used during the computation of the frame clear paths.

5. RESULTS

We have successfully manipulated a variety of biological particles, including mammalian cells (mouse thymocytes, spleen cells, and cultured fibroblasts, rat erythrocytes and alveolar macrophages, and human erythrocytes), plant protoplasts, and Chinese hamster ovary (CHO) chromosomes. In all these experiments, the laser power output was 15 mW, and no damage to the particles was observed. Further experiments are required to determine the effect of optical trapping on the functional properties of biological particles.

Figure 6 shows a side view of a trapped and levitated rat erythrocyte. The image also contains three other erythrocytes, which lie on the bottom of the chamber. As the imaging system is focused at the level of the trap, the image of the trapped cell (marked "T") appears sharper than those of the other cells. The trapped erythrocyte exhibits a preferential orientation in the optical trap, with its plane parallel to the trapping beams.

Figure 7 shows a levitated rat alveolar macrophage (marked "T"), as well as a second macrophage which lies on the bottom of the

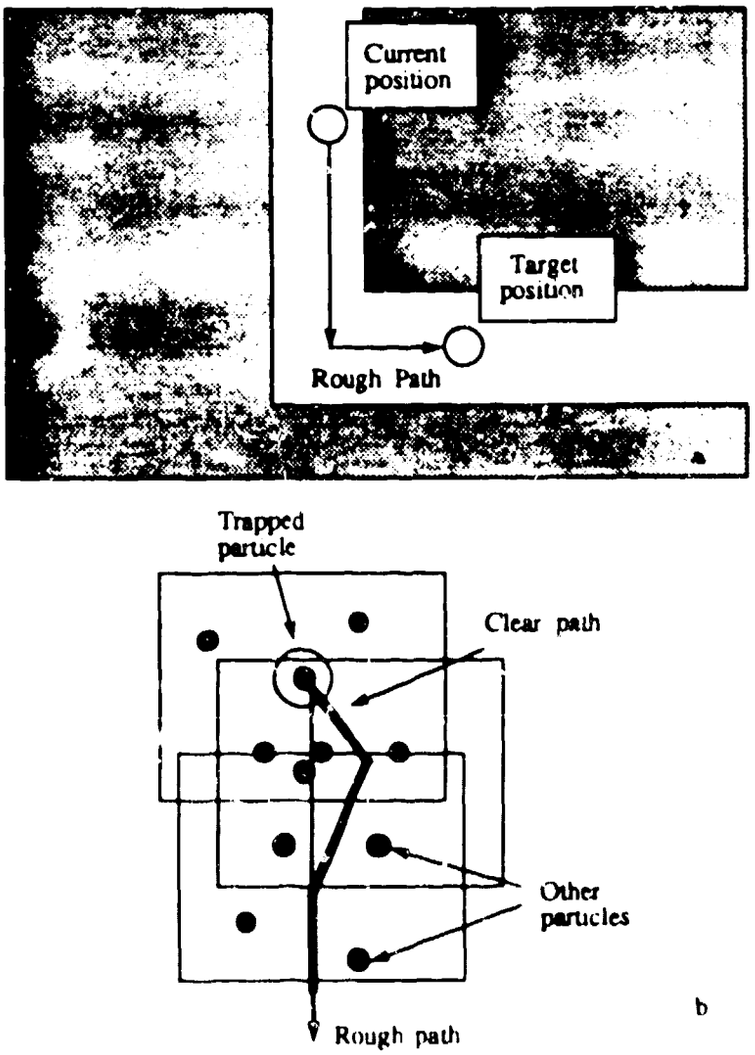

Figure 5 Definition of the rough path (a), and of the clear path (b)

manipulation chamber.

Figure 8 demonstrates the ability of the instrument to trap and position small chromosomes with very high accuracy. This figure shows a chromosome suspension and a 7.5μm polystyrene microsphere. The chromosome marked "1" in Fig. 8a was trapped and placed on the microsphere. The new position of this chromosome is shown in Fig. 8b. A previously deposited chromosome, marked "2", can also be seen in the two images. It is significant that small biological objects such as chromosomes can be placed at selected locations on an object only 7.5μm in diameter.

6. BIOLOGICAL APPLICATIONS

Although optical trapping instrumentation is rapidly developing, the biological applications of optical trapping are still in their infancy. In spite of this, the range of potential applications is impressive. These applications can be grouped in several categories, depending on the particular capabilities of the optical trapping instrument that they emphasize.

6.1. Separation with Absolute Purity

The main application in this category is the separation of rare cells and chromosomes, especially when biochemical and/or genetic analysis is to be performed on the separated particles. The imaging and machine vision capabilities of the instrument allow it to scan a relatively large number of particles, identify the ones of interest, and transport these inside the optical trap to a separate compartment. Furthermore, the instrument can avoid picking up unwanted particles off the bottom of the manipulation chamber, and can monitor the contents of the trap in order to discard unwanted particles that may have drifted into the trap.

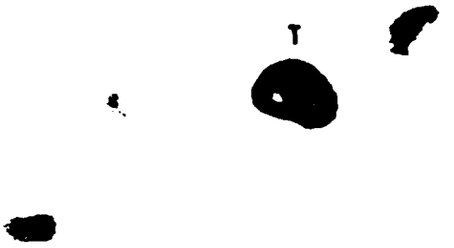

Figure 6. Optical trapping of rat erythrocytes. The trapped cell (marked "T") is roughly perpendicular to the plane of the image.

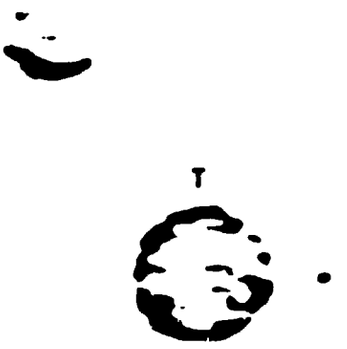

Figure 7. Optical trapping of a rat alveolar macrophage. The cell marked "T" is in the trap, while the one near the top of the image lies on the bottom of the chamber.

6.2. Accurate Positioning

The ability of the instrument to position cells and chromosomes with micron accuracy opens up many applications, such as controlled cell fusion and transfection, and the study of cell-cell and cell-substrate interactions. In such applications, the instrument's positional accuracy will be complemented by its ability to image and monitor cells and chromosomes, and to maintain sample sterility and physiological conditions over extended periods of time.

6.3. Other Applications

The same ability of the instrument to position accurately cells and chromosomes and to move them through microscopic compartments and channels may find important applications in the biochemical and genetic analysis of single cells and chromosomes. Thus, it is our view that the current instrument will evolve into an integrated micro-

scopic laboratory that will combine morphometric analysis and fluorescent labelling measurements not only with cell and chromosome separation, but also with capabilities for biochemical analysis at the level of the single biological particle.

Apart from its many potential uses in terrestrial laboratories, such an instrument would be particularly useful in space biology research. Indeed, the constraints of a space-based laboratory (microgravity, enclosed environment, limited instrumentation weight and size, limited power, requirements for extensive automation and remote control) can be met much more easily by a robotic instrument based on optical trapping than by other types of laboratory instrumentation. We have completed, under NASA sponsorship, the conceptual design for such an instrument [12].

7. CONCLUSIONS

Optical trapping is finding in cell biology and cytogenetics a field of application that is both wide and interesting. Although we are still in the stage where instrumentation is being developed, the combination of optical trapping on the one hand, and robotic control and machine vision on the other, promises to open up completely new and exciting avenues in basic and applied biology, as well as in biotechnology.

8. ACKNOWLEDGMENTS

This work was supported in part by LANL ISRD award X86C and by NASA contract T-1196P. The authors wish to express their gratitude to John C. Martin, Paul Jackson, Renu Kowluru, Bruce Lehnert, and Mike Comforth (all from the Life Sciences Division at LANL), who have contributed advice and/or biological samples.

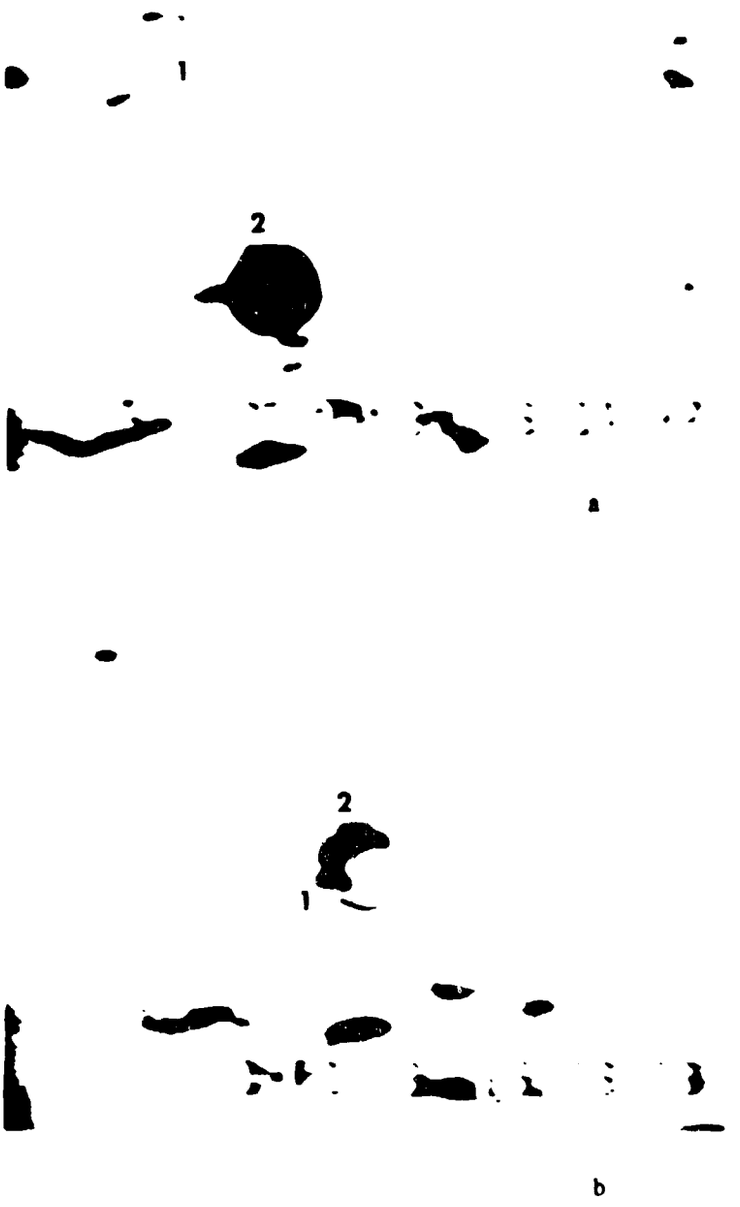

Figure 8. Optical manipulation of CHO chromosomes. The chromosome marked "1" is moved from its original location (a) onto a 7.5 μm polystyrene microsphere (b). The chromosome marked "2" had already been placed on the microsphere.

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